Other anhydride derivatives of glycollic acid may be formed but will not be discussed here.

It has been shown above that the method of preparation of glycollic acid is rather flexible and requires very little attention. The main advantage is that the time-consuming fractional crystallizations can be avoided by this method. It was found by experience that the method possesses no apparent advantage for the preparation of α-hydroxybutyric acid from α-bromobutyric acid.

CHICAGO, ILL.

[HARRIMAN RESEARCH LABORATORY, THE ROOSEVELT HOSPITAL, NEW YORK.]

LEUCINE ANHYDRIDE, A PRODUCT OF THE WATER HYDROLYSIS OF PROTEIN AT HIGH TEMPERATURES.

BY S. S. GRAVES AND J. T. W. MARSHALL, with the assistance of H. W. ECKWEILER.

Received November 1, 1916.

The hydrolysis of protein has been of interest for nearly a century but with the more exhaustive results obtained by acid, alkali and enzyme digestions, the original method of heat hydrolysis with water has practically fallen into disuse. Recently, however, an ether-soluble crystalline substance[1] has been noted among the products of protein hydrolyzed at high temperatures with water.

With the idea of identifying and studying this substance, its preparation and purification were undertaken. Preliminary experiments showed that small amounts of the substance could be prepared by autoclave digestion of protein with water, and that by prolonged heating at high temperatures, the yield was markedly increased. Several grams were thus obtained by the ether extraction of hydrolyzed casein. The crystals from the ether residue were contaminated by a yellowish oil, which was removed by repeated crystallizations from boiling acetone and the substance was finally obtained in the form of fine white needles which melted at 272° (corr.) and sublimed unchanged. Further attempts at purification did not change the melting point nor was it possible to isolate any other crystalline substance from the ether extract. The solubility of the purified substance was found to be 0.3% in acetone at 20°, 0.6% in boiling acetone, 1.4% in chloroform at 20° and its solubility in ethyl and methyl alcohol and in glacial acetic acid was of about this order, while in ether it was very much less. In cold water the pure substance was practically insoluble.

Contrary to expectations the substance was found to have a slight optical activity.

0.2662 gram of substance in 10 cc. of glacial acetic acid.
$a_D = +0.151°$ in 2 dm. tube by Na light.
$[\alpha]_D^{20} = +2.83°$.

[1] Private communication from Dr. S. R. Benedict.

The above physical properties suggested that the substance might be leucine anhydride.

0.1096 g. of subst. gave 0.0137 g. N; 0.1996 g. gave 0.4614 g. CO_2 and 0.1821 g. H_2O.

Calc. for $C_{12}H_{22}N_2O_2$: C, 63.66%; H, 9.80%; N, 12.39%. Found: 63.38%, 10.13% and 12.52%.

Further proof of its identity with leucine anhydride was obtained from melting point determinations of mixtures of this substance with synthetic leucine anhydride[1] and with *l*-alanyl-*d*-alanin.[1]

Substance.	Melting point (corrected).
Leucine anhydride (synthetic)	271°
Leucine anhydride (casein)	272°
l-Alanyl-*d*-alanin	266°
Leucine anhydride (synthetic) + leucine anhydride (casein)	266°
Leucine anhydride (synthetic) + *l*-alanyl-*d*-alanin	220°
Leucine anhydride (casein) + *l*-alanyl-*d*-alanin	224°

From these experiments it seems evident that the ether-soluble crystalline substance prepared from protein (casein) by water hydrolysis at high temperatures is leucine anhydride.

The presence of leucine anhydride among the cleavage products of protein hydrolysis was first mentioned by Bopp in 1849.[2] After digesting casein with 25% H_2SO_4 for a day, he allowed a portion of the sirup to stand for two months and then found that it contained a new crystalline substance which was later identified as leucine anhydride. Salaskin[3] in 1901 isolated this anhydride from oxyhemoglobin by peptic and tryptic digestion for 30 days at 38°. In 1903 Abderhalden[4] reported 9 g. of leucine anhydride from the HCl digestion of 1000 g. of oxyhemoglobin and again in 1907[5] he obtained 0.75% of this anhydride from the hydrolysis of casein by H_2SO_4 and also by HCl.

Abderhalden regards leucine anhydride as a secondary product of leucine and holds that Salaskin's isolation of this anhydride from protein by peptic and tryptic digestion does not with certainty disprove this point. Although heating with acid has been used as a means of preparation of dipeptides from their anhydrides, Abderhalden subsequently made some experiments on the possibility of a reversal of this process. He found that after 16 hours digestion with 5 parts of 25% H_2SO_4, 1 g. of leucyl-leucine yielded a very small amount of anhydride. He thinks, however, that from more complicated mixtures of polypeptides, anhydrides might be more readily obtained.

With the possibility of throwing light on the source of leucine anhydride

[1] Prepared by A. H. Koelker.

[2] *Ann. Chem. Pharm.*, **69**, 16 (1849).

[3] *Z. physiol. Chem.*, **32**, 592 (1901).

[4] *Ibid.*, **37**, 484 (1903).

[5] *Ibid.*, **53**, 19 (1907).

in the heat hydrolysis experiments, weighed amounts of leucine and leucyl-leucine, in separate flasks, were heated with water for 8 hours at 180–200°. On subsequent extraction with ether only a trace of anhydride was found in leucine while from leucyl-leucine more than 90% of the theoretical yield was obtained. From a control experiment with leucine anhydride heated under similar conditions practically the entire amount was recovered.

The above experiments would appear to eliminate the possibility of the building up of the anhydride from any leucine separated by the hydrolysis of protein. If, however, the dipeptide, leucyl-leucine, were among the cleavage products of the protein, it may readily be seen that this dipeptide would go over almost quantitatively to the anhydride. At the same time, there remains the possibility of the anhydride existing as such in the protein molecule as was indicated by Salaskin's experiments.

Since the heat hydrolysis method apparently gives results which may be interpreted either as measure of the leucyl-leucine or of the leucine anhydride content of the protein molecule, the amount of anhydride from several proteins has been determined quantitatively and the results seem worthy of note.

In each case 25 g. of protein and 200 cc. of H_2O were heated in an autoclave at 180–200° for 16 hours. The brown syrupy liquid which resulted was extracted with ether in a Soxhlet apparatus for 16 hours; the ether was then removed by distillation and the residue purified by repeated crystallizations from hot acetone up to the point at which a saturated solution of the anhydride was but slightly yellow. The purified substance formed from 15 to 20% of the crude ether extract. The crystals were finally dried at 100° and weighed after cooling in a desiccator.

Protein.	Leucine anhydride.	Protein.	Leucine anhydride.
Casein	1.5%	Witte's peptone	1.0%
Egg albumin	1.2%	Silk	0.09%
Edestin	1.2%	Gelatin	0.04%

NEW YORK, N. Y.

[CONTRIBUTION FROM CHEMISTRY SECTION OF THE IOWA AGRICULTURAL EXPERIMENT STATION.]

AN IODINE ADDITION PRODUCT OF CUMARIN.

BY ARTHUR W. DOX AND W. G. GAESSLER.

Received October 25, 1916.

The recent work of Emery and Palkin[1] on organic periodides is of especial interest to the writers in view of some experiments which we began two years ago on the reaction that takes place between Wagner's reagent and a solution of cumarin. Upon the addition of a few drops of an aqueous solution of iodine and potassium iodide to an aqueous solution of cumarin,

[1] THIS JOURNAL, 38, 2166 (1916).